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Lepiota himalayensis (*Basidiomycota, Agaricales*), a new species from Pakistan

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Abstract — A new *Lepiota* species from the Himalayan moist temperate forests in Pakistan is described and illustrated. The orangish-brown basidiocarp with dark blackish scales on the pileus, ellipsoid spores, narrowly clavate to clavate cheilocystidia, and the narrowly clavate to clavate nature of trichodermial elements of pileal covering are striking features of this species. The phylogenetic relationship with related species based on ITS-rDNA sequences is discussed.

Keywords — lepiotaceous fungi, mushroom diversity, phylogeny, rDNA

Introduction

The Himalayan moist temperate forests of Pakistan are distinguished by the luxurious vegetation of conifers and deciduous trees. In these forests, located at elevations of 1370–3050 m, maximum summer temperatures vary from 10.7–18°C, rainfall averages 59.3 cm, and humidity ranges up to 57% (Champion et al. 1968). Most of the mushrooms are still to be identified, even though Himalaya is one of the twenty-five world biodiversity hotspots (Myers et al. 2000).

Lepiota (Pers.) Gray (*Agaricales, Basidiomycota*) is an important and diversified genus comprising more than 400 species (Kirk et al. 2008, Liang &Yang 2011). This genus is characterized by a scaly pileus, free lamellae, partial veil in the form of annulus, a universal veil, and smooth, white, dextrinoid spores; most species have clamp connections (Vellinga 2001, Kumar & Manimohan 2009). Lepiotaceous fungi are very common in the forests of Pakistan, and of the 21 *Lepiota* species thus far reported from this country (Ahmed 1980, Shibata 1992, Murakami 1993, Sultana et al. 2011), 12 species are known from its Himalayan moist temperate forest (Ahmed et al. 1997, Sultana et al. 2011). During fieldwork in this area, a new species of *Lepiota* was collected from a

forest dominated by *Abies pindrow* (Royle ex D. Don) Royle. We describe it here as new and compare it with related species.

Materials & methods

Basidiocarps were carefully dug with the help of a knife and photographed in the field. Collected material was characterized morpho-anatomically and molecularly. Basidiocarp descriptions use the terminology of Vellinga (2001) and colors were determined and coded according to Anon (1975). Basidiocarp sections were stained with Congo Red and Melzer's reagent. Twenty-five basidiospores and 20 each of basidia, cheilocystidia, pileal elements, and stipe elements were measured from one basidioma. Abbreviations include: avl = average length, avw = average width, Q = length / width of basidiospore. Drawings were made using a camera lucida attached to the compound microscope. The dried specimen was deposited in the LAH Herbarium, Department of Botany, University of the Punjab, Lahore.

For molecular analysis, dried material was ground in liquid nitrogen, placed in 2% CTAB buffer, and DNA was extracted according to Porebski et al. (1997). The rDNA ITS regions were amplified using universal primer pair ITS1F and ITS 4 (White et al. 1990, Gardes & Bruns 1993). PCR was performed in 25 µL reaction volumes according to Gardes & Bruns (1993). PCR product of the ITS-amplified region containing ITS-1, 5.8 and ITS-2 was directly sequenced in both directions using the same pair of amplification primers (Macrogen, Korea). Nucleotide sequence comparisons were performed with Basic Local Alignment Search Tool (BLAST) network services using National Center for Biotechnology Information (NCBI), USA database. For further phylogenetic analysis and alignment of sequence, closely related sequences were retrieved from GenBank. Sequence alignments and phylogenetic analysis were performed using Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2011). Maximum Likelihood (ML) method was based on the Jukes-Cantor model of nrITS sequences using Nearest-Neighbor-Interchange (NNI) as ML heuristic search method. Phylogeny was tested by bootstrap value of 1000 replicates. Nucleotide sequence of *L. himalayensis* was submitted to European Molecular Biology Laboratory (EMBL) database and is available in GenBank (HE614898).

Taxonomy

Lepiota himalayensis Razaq & Khalid, **sp. nov.** Figs 1, 3

Mycobank mb 563941

Differs from *Lepiota farinolens* by its larger basidiocarps, its smaller basidiospores, and its narrowly clavate to clavate cheilocystidia.

Type: Pakistan, Khyber Pakhtunkhwa, Himalayan Moist Temperate Forests, Khanspur, at 2250 m a.s.l., solitary, on moist ground under *Abies pindrow*, 23 August 2010, Abdul Razaq KP-63 (**Holotype**, LAH 230810; GenBank nrITS sequence HE614898).

Etymology: The specific epithet refers to the general collection area of the type.

Pileus 4 cm diam., campanulate to plano-convex, cinnamon (5.2YR/5.4/7.7) to umber (7.OYR/3.7/5.8) with fibrillose surface and scaly covering; scales dark

Fig. 1: *Lepiota himalayensis* A. Basidiocarp with prominent pileus scales. B. Reverse side of basidiocarp showing white lamellae. Scale Bars: A = 2 cm; B = 1.5 cm.

brown to blackish; central disc obtuse to slightly umbonate, distinguished from rest of pileus having black and prominent scales that are more or less uplifted and prominent; margins dentate, at maturity fragile and broken; context moderately thick, white. Lamellae free, crowded, white to cream (10YR8/4), two tiers of lamellulae alternating with lamellae. Stipe 6.0 × 0.6 cm, centrally attached, cylindrical and slightly tapering towards apex, orange-brown (7.5 YR 5/6; 5 YR4–5/6); surface scaly, scales brown and more prominent on lower part of the stipe below the annulus, above annulus smooth; with white hyphal mass at the base, annulus rudimentary, non-persistent. Odor none. Taste not recorded.

Basidiospores 6.7–8.3 × 3.0–4.0 µm, avl × avw = 7.4 × 3.5 µm, Q = 2.0–2.2, oblong, internal contents greenish, brown in Melzer's reagent. Basidia 23.5–27.7 × 8–9.7 µm, clavate to tapering towards base, with oil-like contents. Pleurocystidia absent. Cheilocystidia hyaline, narrowly clavate to clavate, 19–28 × 5–10.5 µm. Pileus covering a trichodermium with erect or ascending septate terminal elements, 53.5–99.5 × 2.5–7.5 µm, narrowly clavate to clavate without any shorter elements at the base of longer ones, light brown to hyaline. Stipe covering a cutis with occasional trichodermial patches of clavate terminal elements 42.5–127.5 × 8.0–9.0 µm, hyaline. Clamp connections present.

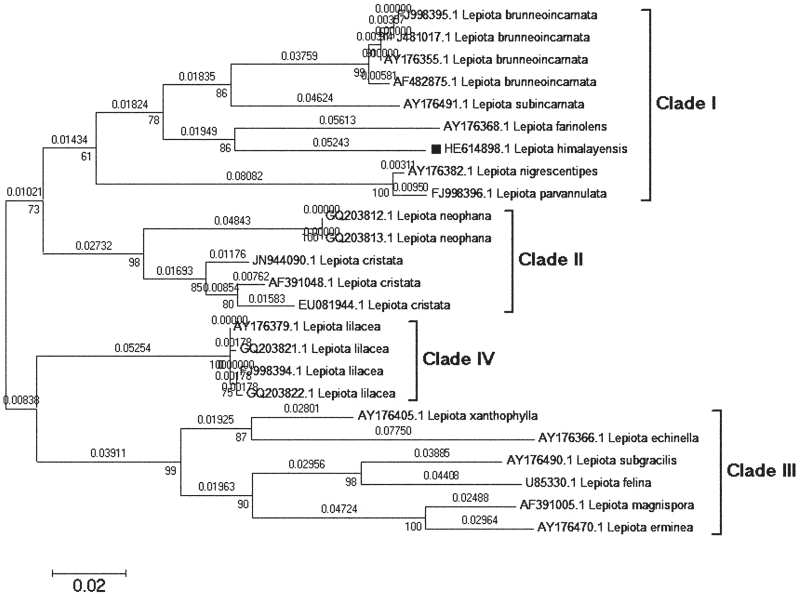

Fig. 2: Phylogenetic relationship of *Lepiota himalayensis* with other members of *Lepiota* based on Maximum Likelihood method inferred from nrITS sequences. Bootstrap values based on 1000 replicates are shown below the branches and below 50 are not shown. Branch length is shown above each branch. The topology of the tree based on Maximum Parsimony is same for *Lepiota himalayensis*. The analysis involved 30 sequences. All positions containing gaps and missing data were eliminated. There were a total of 649 positions in the final dataset.

Molecular description and phylogenetic analysis

When amplified, the target region of the fungal genomic DNA of *Lepiota himalayensis* generated fragments of approximately 750bp. Initial BLAST sequence analysis revealed a maximum sequence match with *L. brunneoincarnata* Chodat & C. Martín (GenBank FJ481017, FJ998395, AY176355). The top 100 sequence matches in the BLAST analysis belong to *Lepiota*, of which the topmost 35 sequences belonging to different *Lepiota* sections were included in the phylogenetic analysis.

An initial phylogenetic analysis shows that *L. himalayensis* clusters among sequences belonging to *Lepiota* sect. *Ovisporae* (J.E. Lange) Kühner. Additional published sequences from sects. *Ovisporae* and *Lepiota* were included in the alignment. In the maximum likelihood method (Fig. 2), four clades are formed, all members of sect. *Ovisporae* without short elements at the base of the long cells in the pileus covering clustered together in clade I. In this clade *L. farinolens* Bon & G. Riousset is closely related to *L. himalayensis*. *Lepiota*

subincarnata J.E. Lange and *L. brunneoincarnata* cluster together as a sister clade to *L. himalayensis* and *L. farinolens*. All taxa in this clade are distinguished by having only long pileal elements. *Lepiota parvannulata* (Lasch) Gillet and *L. nigrescentipes* G. Riousset form a sister clade to sect. *Ovisporae*. Additionally, taxa with a pileal covering composed of longer elements with shorter elements at the base of the longer ones cluster together in a separate clade (Fig. 2, Clade III), while taxa with a hymeniform pileal covering cluster together in Clade II. All sequences of *L. lilacea* Bres. form the separate clade IV. According to our phylogenetic analyses, the close relatives of *L. himalayensis* are *L. farinolens*, *L. subincarnata,* and *L. brunneoincarnata.*

Discussion

Lepiota himalayensis is characterized by orange-brown basidiomata with dark black squamules on the pileus, a trichodermial pileus covering composed of light brown to hyaline and elongate clavate elements, oblong spores, and narrowly clavate to clavate cheilocystidia. *Lepiota himalayensis* can be placed in *L.* sect. *Ovisporae* based on the trichodermial pileal covering and oblong basidiospores shorter than 10 µm (Singer 1986, Vellinga 2001, Kumar & Manimohan 2009).

Occurrence of non-persistent annulus and scales on lower part of the stipe bring *L. himalayensis* close to *L. helveola* Bres. and *L. pallidiochracea* J.F. Liang & Zhu L. Yang. However, the grayish red to reddish stipe and the two types of elements in *L. helveola* (Kosakyan et al. 2008) distinguishes that species from *L. himalayensis.* Although the basidiospores are similar in size (7–8 × 3.5–4 µm in *L. helveola*), the colourless spores in *L. helveola* contrast with the greenish colour in our species. Liang & Yang (2011) described variable cheilocystidia and apically attenuate pileal elements for *L. pallidiochracea* that distinguish it from *L. himalayensis.*

Four distinct clades were formed in the phylogenetic analysis. Clade I comprises sect. *Ovisporae* plus *L. parvannulata* and *L. nigrescentipes*. The position of these two species was not clear in Vellinga's (2003) phylogenetic tree. *Lepiota himalayensis* groups with *L. brunneoincarnata*, *L. subincarnata*, and *L. farinolens* in our maximum likelihood nrITS analysis (Fig. 2, Clade I) and lies in Vellinga's clade 2. The trichodermial pileus coverings of *L. brunneoincarnata* and *L. himalayensis* are comparable but the spore size (8–10.5 × 4–6 µm) and vinaceous blackish brown pileus colour distinguishes *L. brunneoincarnata* (Courtecuisse 1999). In the same clade *L. subincarnata* is separated by its orange-white pileus and smaller spore size (4.5–7.5 × 3–5 µm) (Kumar & Manimohan 2009). Molecular analysis showed a close relationship between *L. farinolens* and *L. himalayensis* but they differ morphologically. According to Salom & Siquier (2001), *L. farinolens* has smaller basidiocarps (15–25 mm), larger basidiospores (7.2–9.6(–11.2) × 4.8–5.4(–6) µm), variably

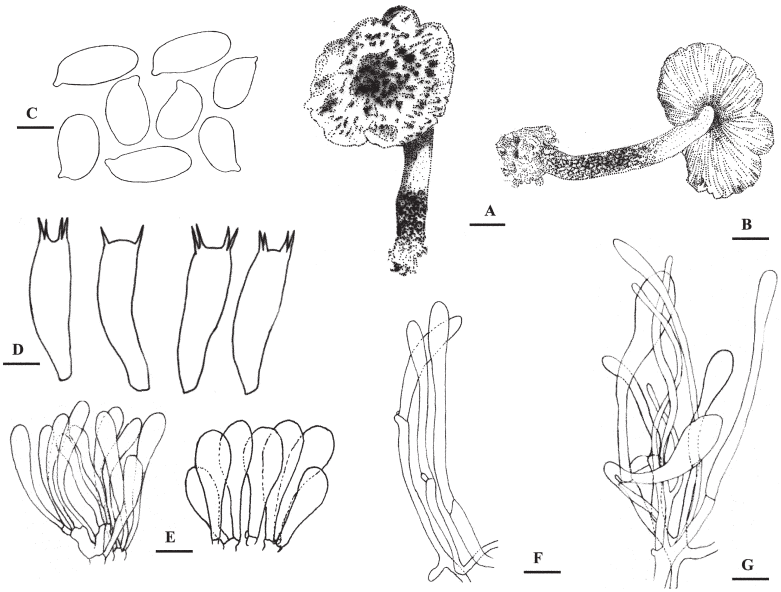

Fig. 3: *Lepiota himalayensis*. A–B. Basidiocarp. C. Basidiospores. D. Basidia E. Cheilocystidia. F. Stipe covering. G. Pileal covering elements. Scale bars: A–B = 1 cm; C = 3.5 µm; D = 6 µm; E = 13 µm; F = 21 µm; G = 16 µm.

shaped (cylindrical claviform, fusiform) cheilocystidia, and a single type of pileal element with thickened apices. Clade III contains those *Lepiota* species of sect. *Ovisporae* and sect. *Lepiota* that are characterized by longer pileal elements with an under-layer of shorter ones. Vellinga (2003) recognized these species as belonging to a polyphyletic clade containing species with fusiform, 'sphenisciform', and oblong spores.

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